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Original Article

Digging deep into molecular pathways: investigating the effects of 9S-HOD on leukemia cells using a systems biology approach

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Abstract

Background: Acute myeloid leukemia (AML) is a malignant disorder characterized by a poor prognosis. Current therapeutic approaches include chemotherapy, steroids administration, and blood transfusion. Previous studies have highlighted the potential anticancer property of 9-hydroxyoctadecadienoic acid (9S-HOD). This molecular computational research aims to investigate the intricate molecular mechanism underlying the effects of 9S-HOD on leukemia cells.

Methods: Utilizing proteomic data and the optimum numbers of the first neighbors from the STRING database, Cytoscape 3.9.1 along with its applications, NetworkAnalyzer and ClueGO+CuePedia were employed to analyze the constructed protein-protein interaction (PPI) network, its centrality and enrichments.

Results: The analysis identified five proteins namely ACTB, HSP90AA1, GAPDH, TP53, and HSP90AB1 as potential central nodes within the PPI network. Furthermore, gene ontology analysis revealed "Response to salt stress" and "Positive regulation of type 1 interferon production" as enriched biological processes associated with these key elements of the PPI network. HSPA8, MYC, and KAT5 were identified as seed proteins within the sub-networks.

Conclusion: The findings suggest that the effect of 9S-HOD on the leukemia cells primarily involves the regulation of ACTB, HSP90AA1, HSP90AB1, GAPDH, and TP53. additionally, HSPA8, MYC, and KAT5 emerged as important proteins influenced by 9S-HOD.

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1. Introduction

The investigation of the molecular mechanisms underlying acute leukemia, a heterogeneous childhood disease is of utmost importance. The manifestation of this malignancy in B and T Cells varies depending on the age of individuals (1). The incidence of new leukemia cases continues to rise annually, with approximately 6000 new cases reported (2). Current treatment approaches for leukemia include chemotherapy, steroids administration, and blood transfusion (3, 4). however, these treatments often yield unfavorable outcomes and are accompanied by so various side effects. Additionally, the development of chemotherapy resistance poses further challenges in cancer therapy (5). Furthermore, the genetic heterogeneous of leukemia makes diagnosis and prognosis difficult (6, 7). In light of these challenges, alternative approaches, such as the utilization of natural agents derived from plants and animals, have shown promising cytotoxicity properties (8).

9-hydroxyoctadecadienoic acid (9S-HOD) derived from linoleic acid, has demonstrated potential as an effective natural antitumor agent (9, 10). Understanding the mechanisms underlying cancer initiation, development, and treatment response often involves bioinformatic approaches, such as PPI network analysis (11). In this study, the dataset employed for the bioinformatics analysis was obtained from proteomic research conducted on HL-60 cells treated with 9S-HOD (10). Through complementary molecular analysis, dysregulated proteins that significantly contribute to the stability and robustness of the PPI network can be identified as promising biomarkers. Furthermore, this method allows for monitoring the treatment's molecular-level effects. In the context of cancer research, proteins that exhibit regulation in the presence of the treatment and occupy central positions within the PPI network may represent key components of the treatment's anticancer mechanism. These molecules can serve as indicators of how the treatment modulates processes within cancer cells (12). The identification of central nodes within the PPI network is achieved by assessing key parameters such as degree (K) and betweenness centrality (BC) (13). Given the significant side effects associated with existing therapeutic methods for leukemia, exploring natural sources at the molecular level becomes crucial.

In this regard, investigating the molecular mechanisms and antitumor activity of 9S-HOD on leukemia cells is of great importance. investigating the molecular mechanisms and antitumor activity of 9S-HOD on leukemia cells is of great importance.

2. Methods

2.1. Data collection

In the main research, the proteome analysis of the HL-60 cells as a leukemia cell line exposed to 9S-HOD (20 μM), was conducted using 2DE based proteomics (10). The analysis identified 20 protein spots using MALDI-TOF-MS. Among these, 16 protein

identifiers were selected for PPI network analysis. The study focused on evaluating the PPI network and gene ontology but did not the hub-bottleneck proteins and the first neighbors of the queried individuals.

2.2. Network analysis

Hub-bottleneck identification involves analyzing central proteins in the PPI network. Hubs are highly connected nodes in the network, while bottlenecks act as bridges between other nodes.

Hubs have high degrees, indicating numerous connections, while bottlenecks have high BC, signifying their role in connecting different parts of the network. Hub bottlenecks are crucial for the stability and strength of the PPI network (14).

To visualize the PPI network in the study, Cytoscape 3.9.1 (https://cytoscape.org/) and the STRING database v.2 (http://string-db.org/) were utilized. Cytoscape is an open-source software platform for visualizing complex networks and integrating attribute data. STRING is a functional protein association database that provides protein-protein interaction networks and functional enrichment analysis (15-17).

By using Cytoscape and the STRING database, the researchers were able to visualize and analyze the PPI network in their study, gaining insights into the interactions between the identified proteins. In the PPI network analysis, the STRING App was used to query proteins based on different sources. However, since the nodes in the network had poor connectivity, an additional 50 first neighbors were added to the queried proteins to enhance the network's connectivity. The network was then analyzed using the Network Analyzer application to identify the hub-bottlenecks. Hub-bottlenecks are proteins with high connectivity and act as central nodes in the network. To further analyze the network, subnetwork analysis was performed using MCODE v.2. This analysis aimed to identify dense regions or clusters within the PPI network. Proteins within these complexes that exhibited high connectivity were referred to as seed proteins (18). By applying these criteria, the researchers identified and analyzed the protein complexes or clusters within the PPI network, focusing on proteins with significant connectivity and central roles in the network.

Based on the information provided, it seems that ClueGO 2.5.9 and CluePedia 1.5.9 were used for enrichment analysis of hub bottlenecks with a focus on biological processes (BPs). (19, 20). Biomarkers associated with these terms were also analyzed. The analysis utilized different layouts for visualizing the ontology including pathway view, bar view, and pie chart view. In this case, a pie chart view was selected.

2.3. Statistical analysis.

The default confidence score cutoff of 0.3 was applied to construct the PPI network.

The statistical criteria used for the protein complexes analysis were as follows:

Degree cutoff: Proteins with at least 2 connections within the complex were considered.

Node score cutoff: Proteins with a score of 0.2 or higher were included in the analysis.

K-core: Proteins with a K-core value of 2 or higher were considered for the analysis.

The statistical analysis of the examination was based on the following criteria:

- ✓ Term grouping: 0.5
- ✓ Number of proteins per term: 2
- ✓ Percentage of proteins per term: 3
- ✓ P-value of grouping: 0.05
- ✓ Correction test: Bonferroni step-down

These criteria were likely used to determine the significance of the enriched terms and groups in the biological process analysis.

3. Results

The PPI network analysis was conducted with a confidence cutoff of 0.3. In the first network query, approximately 71% of the nodes were found to have direct connections, while the remaining nodes remained as individual nodes, meaning they were not directly connected to the main network. The visualization of this first network query is depicted in **Figure 1**.

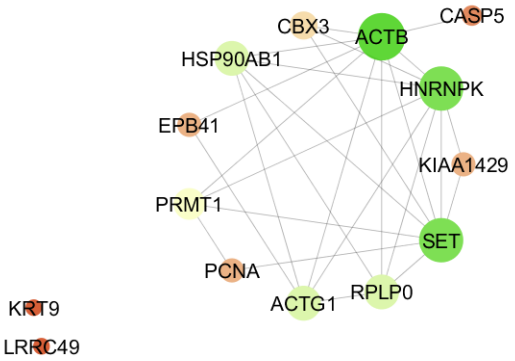

Figure 1. Protein-protein interaction network of treated HL-60 cells with 9S-HOD. A confidence score cutoff of 0.3 is considered.

Continuing the PPI network analysis, a second network query was performed, resulting in a network with 64 nodes and 735 connections (**see Figure 2**). However, the visualization of this second query is not shown in the provided information. In this network, two DEPs (Differentially Expressed Proteins), namely LRRC49 and KRT9, did not connect to the main network.

To gain a better understanding of the underlying mechanism of (9S-HOD) in leukemia, a centrality analysis of the PPI network of the HL-60 proteome was conducted. The "Centrality Analyzer" was used to measure the hub bottlenecks in the network. **Table 1** lists the results of the centrality analysis, specifically the highest-ranked degree and betweenness centrality values.

Based on this topological examination, 10% of the proteins with the highest ranked degree and betweenness centrality values were assigned as hub bottlenecks, indicating their potential importance in the network.

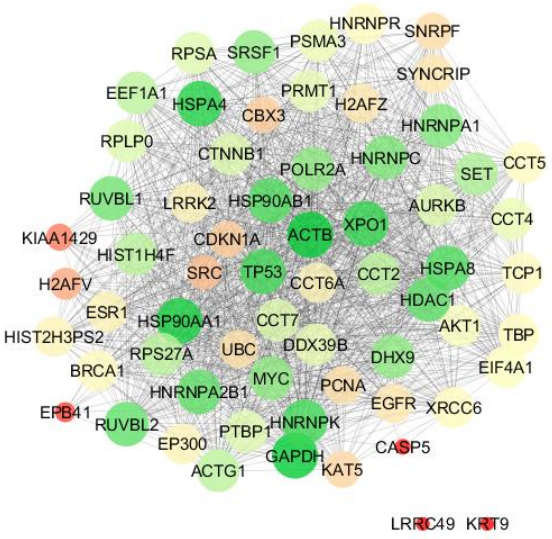

Figure 2. Protein-protein interaction network including 14 queried proteins plus 50 added first neighbors of treated HL-60 cells with 9S-HOD. A confidence score cutoff of 0.3 is considered.

Table 1. The hub-bottlenecks of the PPI network ranked based on degree value; the asterisk sign indicates the queried DEPs

Row	Display name	K	BC
1	ACTB*	50	0.08
2	HSP90AA1	45	0.04
3	GAPDH	44	0.04
4	TP53	43	0.04
5	HSP90AB1*	37	0.03

Based on the provided information, the PPI network analysis identified ACTB as the most valued hub bottleneck with a K of 50 and BC of 0.08. HSP90AA1 ranked second with a degree of 45 and betweenness centrality of 0.04. Notably, two DEPs, ACTB and HSP90AB1, are among the hub bottlenecks. ACTB is up-regulated, while HSP90AB1 is down-regulated under the treatment influence in leukemia. To locate protein clusters within the PPI network, the MCODE

plugin was used. This analysis identified three protein complexes, as shown in **Figure 3**. The first cluster is centered around the seed protein HSPA8, the second cluster is associated with MYC, and the third sub-network involves KAT5.

Enrichment annotation was performed to gain further understanding of the hub-bottlenecks in the PPI network. The relevant BPs were identified for the key nodes of the PPI network via gene ontology analysis. The two identified classes of BPs are "Response to salt stress" and "Positive regulation of type 1 interferon production" including 60 and 40 percent of the terms respectively. These BPs provide insights into the potential functional roles of the key nodes in the context of the PPI network. Based on the provided information, a PPI network analysis was performed using Cytoscape and the STRING database plugin. Initially, 16 out of 20 DEPs spots were queried, resulting in a network with 14 nodes and 17 connections. It appears that no first neighbors were added in this query.

4. Discussion

In order to gain a deeper understanding of how 9S-HOD affects Leukemia, researchers conducted an original proteomics study to analyze the differentially expressed proteins resulting from this treatment (10). It is crucial to high-throughput screening techniques to identify variations in proteome profiles between subjects who received the treatment and those who did not (21). To enhance our comprehension of the significance and contributions of biomarkers, it is necessary to utilize a bioinformatics approach that incorporates PPI network analysis, which can offer additional insights into the underlying mechanisms (22). In our study, we specifically focused on the topological features of the protein-protein interaction (PPI) network, namely the degree and betweenness centrality. These features were investigated to identify nodes that play a crucial role in disease monitoring when exposed to the treatment (22). Based on the analysis of degree and betweenness centrality, five proteins were identified as hub bottlenecks in our study: ACTB, HSP90AA1, GAPDH, TP53, and HSP90AB1. while ACTB and HSP90AB1 are among the queried DEPs, the other three central nodes (GAPDH, TP53, and HSP90AA1) were determined as the first neighbors added to the network.

To further elucidate the role of these hub bottlenecks in the anticancer mechanism of 9S-HOD, we conducted an enrichment analysis focusing on the detection of biological processes. Two prominent groups were identified, namely “Response to salt stress” and “Positive regulation of type 1 interferon production”. These biological process terms are significantly associated with the hub bottlenecks of HSP90AA1, TP53, GAPDH, and HSP90AB1.

Overall, our study highlights the importance of these central nodes in the PPI network and their potential involvement in the anticancer effects of 9S-HOD. The enrichment analysis provides further insights into the biological processes related to these hub bottlenecks, shedding light on their functional roles in the mechanism of action.

The significance of seed nodes within protein complexes is pivotal for their interactions within the network. In our analysis, we identified HSPA8, MYC, and KAT5 as crucial nodes or seed proteins in the PPI network. Notably, none of these seed proteins were identified as hub bottlenecks in the PPI network. While hub bottlenecks play a vital role in overall network connectivity and information flow, seed proteins are integral to the formation and stability of protein complexes. The presence of these important seed proteins, along with the hub bottlenecks, suggests their potential involvement in the underlying mechanism of the anticancer properties of 9S-HOD. Their interactions and associations within the network could contribute to the overall functional impact of 9S-HOD on cancer cells.

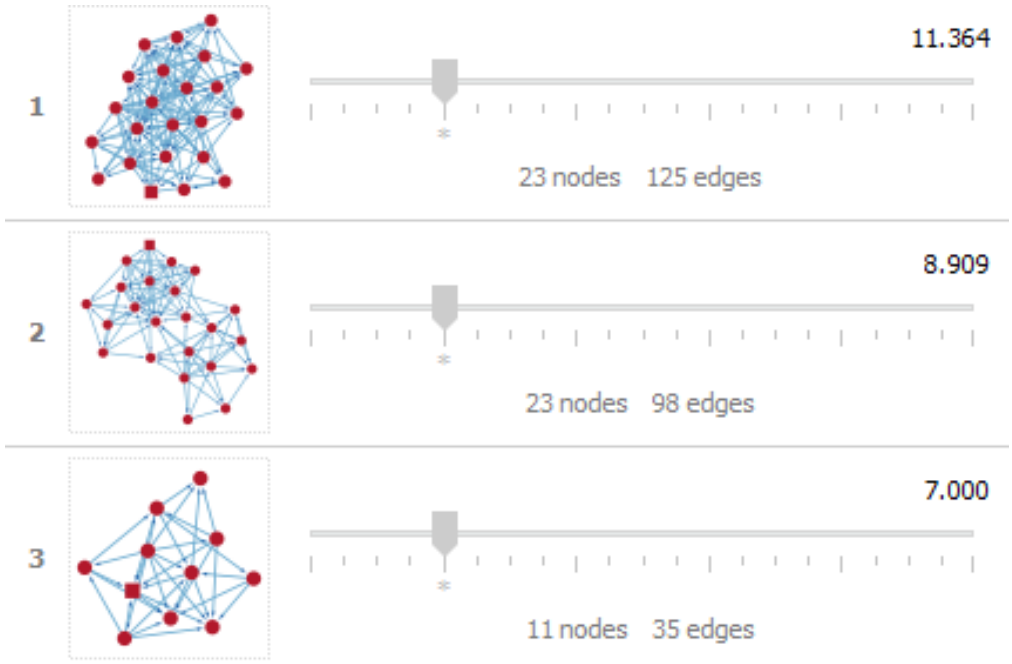

Figure 3. Three ranked protein complexes (densely connected regions), rectangle node in each cluster is the seed protein. Degree cutoff = 2 was considered.

Therefore, the combined presence of hub bottlenecks and seed proteins could be essential in unraveling the underlying mechanisms behind the anticancer properties of 9S-HOD. These two types of proteins play distinct but complementary roles in the dynamics of the network and the biological processes involved. Therefore, examining their involvement in studies on cancer treatment could yield further insights in this context. The first central protein, actin-b (ACTB), belongs to the housekeeping family and has been found to be closely associated with various types of cancers, including leukemia (23). This protein is known to be over-expressed in multiple malignancies and contributes to metastasis progression (23). In the prime study, the over-expression of ACTB was observed in response to the exposure of 9S-HOD. This suggests that over-expression of ACTB is compensated by the other affected proteins such as the hub bottlenecks from the added first neighbors. The Heat Shock Proteins (HSPs) family encompasses various members and plays a fundamental role in eukaryotes. Heat shock protein 90 (HSP90) is particularly significant in the mechanisms of cancer development. This family has been on a focus for drug target therapy in cancer treatment approaches (24). Up-regulation of HSPs in different types of tumors has been reported in numerous studies (25, 26).

Hsp90AA1 is the second-ranked central protein among the added first neighbor proteins. Dysregulation of this protein has been implicated in numerous previous cancer studies (27). Although it was not identified as a significant differentially expressed protein in the original study, further investigations could help uncover its potential contribution to leukemia pathogenesis and treatment. Additionally, Hsp90AB1, another member of the HSPs family of chaperones, is the fifth protein in the highly ranked proteins in **Table 1**. This protein was found to be differentially expressed in the original

study, with statistically significant down-regulation observed in the treated subjects (10). The reduction of Hsp90AB1 levels by 9S-HOD suggests its regulatory effects in cancer therapy.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), the next highly ranked protein, is another housekeeping protein involved in the glycolysis process. Its dysregulation in cancers has been frequently reported in various studies (28, 29). It appears that GAPDH, similar to ACTB, is influenced by 9S-HOD to prevent cancer development. This protein tends to be up-regulated in many cancer types (30).

TP53, a tumor suppressor gene, is highly correlated with cancer initiation and development due to its role in the cell cycle (31). Abnormalities in this protein are also associated with leukemia pathogenicity (32). As a hub-bottleneck protein, TP53 holds value in the treatment approaches for leukemia. Based on the literature review of these central nodes, it is evident that hub-bottlenecks and their associated biological processes could play a crucial role in cancer treatment involving 9S-HOD. Additionally, seed proteins can also play a prominent role as target proteins in cancer therapy. HSPA8, a chaperone protein, has been identified in previous studies as contributing to AML (33). MYC, another seed protein, is reported as a differentially expressed protein in AML and is considered a potential target t (34). KAT5, the third seed protein, possesses DNA-repairing properties and is also proposed as a candidate for drug targeting in AML (35).

Targeting these proteins with specific agents like 9S-HOD for drug targeting and expression regulation holds promise. In summary, for therapeutic purposes, the identified potential central proteins could be beneficial as targets for drug targeting in AML, but further proteomics evaluations and complementary studies are necessary to fully understand their roles and validate their efficacy.

In the presence of 9S-HOD, two classes of biological processes, namely "Response to salt stress" and "Positive regulation of type 1 interferon production," were identified as altered in the treated cells. It is worth noting that the significance of interferon regulatory factors in leukemia has been extensively studied and reported by previous researchers (36). These findings suggest that 9S-HOD may influence the cellular response to salt stress and positively regulate the production of type 1 interferons. The involvement of interferon regulatory factors in leukemia indicates their potential relevance in the context of the observed altered biological processes induced by 9S-HOD. Further research and investigations are necessary to elucidate the specific mechanisms underlying the impact of 9S-HOD on these biological processes and how they relate to the pathogenesis and treatment of leukemia.

5. Conclusion

In conclusion, the central dysregulated proteins such as ACTB, HSP90AA1, HSP90AB1, GAPDH, and TP53, along with their associated biological processes, as well as the identified seed proteins HSPA8, MYC, and KAT5, demonstrate potential as suitable drug targets for the 9S-HOD. Apart of the introduced proteins are appeared among the new set of the added first neighbors. It is recommended to conduct further investigations to explore the regulation of these proteins and biological terms as complementary therapeutic approaches. By developing a better understanding of their roles and interactions, it is possible to enhance the anticancer activity of medicines targeting these proteins and biological processes, ultimately advancing leukemia treatment.

Conflict of interest

There is no conflict of interest.

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Ethical approval statement

This project is approved by IR.SBMU.RETECH.REC.1401.763 ethical code.

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